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The stability of docosahexaenoic acid in two *Artemia* species following enrichment and subsequent starvation

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Abstract

Two *Artemia* species (*Artemia franciscana*, GSL strain and a population from *Artemia sinica*, ARC No. 1188) were enriched with two different emulsions containing high levels of docosahexaenoic acid (DHA) (23 and 44% of total fatty acids, respectively) in filtered seawater (30 ppt) at 28°C. After enrichment nauplii were starved for 72 h at three different temperatures (6.1, 12.2 and 22°C). Following enrichment the DHA content and DHA/EPA ratio reached a maximum 41.2 and 42.8 mg g^{-1} dry weight and 1.88 and 2.09 in *A. franciscana* and *A. sinica*, respectively. During starvation at 12.2°C the DHA content in *A. franciscana* decreased steadily throughout the starvation period towards 1.11–2.89 mg g^{-1} dry weight (DHA/EPA ratio of 0.4) after 72 h, whereas in *A. sinica* the DHA content was > 20 mg g^{-1} dry weight (DHA/EPA ratio of 1.7–2). A quantitative reduction of DHA in *A. sinica* was observed only during the first 24 h of the starvation period. The degradation rate of DHA in *A. franciscana* increased with rising temperature (22°C) and decreased at the lowest temperature (6.1°C). This was not the case for *A. sinica*, during starvation at different temperatures the DHA level in *A. sinica* remained at a relatively high and constant level. © 1997 Elsevier Science B.V.

Keywords: *Artemia*; Enrichment; Docosahexaenoic acid; Eicosapentaenoic acid; $(n-3)$ HUFA; Starvation; Essential fatty acid metabolism

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1. Introduction

It is well documented that long chain polyunsaturated fatty acids (PUFA) of the $(n-3)$ series are essential for marine fish (Yone and Fuji, 1975; Watanabe, 1982; Koven et al., 1990; Rainuzzo et al., 1992a), particularly eicosapentaenoic acid [20:5($n-3$), EPA] and docosahexaenoic acid [22:6($n-3$), DHA] (Kanazawa et al., 1979; Léger et al., 1981; Watanabe, 1988; Navarro et al., 1993). In fish and higher vertebrates the phospholipids of neural tissues, specifically brain and retina, are rich in 22:6($n-3$) (Neuringer et al., 1988; Tocher and Harvie, 1988; Bell and Tocher, 1989; Anderson et al., 1990; Bazan and Scott, 1990) and 22:6($n-3$) may have a specific role in neural membranes (Sargent et al., 1993). The utilization of 22:6($n-3$) as an energy source, during early embryogenesis, does occur in larvae of a number of marine species (Falk-Petersen et al., 1989; Tocher et al., 1985; Rainuzzo et al., 1992b), but after yolk sac absorption the larvae are completely dependent on their captured prey for provision of these essential fatty acids (EFA).

Recent research has demonstrated that supplementation of DHA in the live feed of marine fish larvae improved the growth, survival, and stress resistance (Watanabe, 1993), while the DHA/EPA ratio may be an important factor in the pigmentation of several flatfish species (Reitan et al., 1994).

Brine shrimp nauplii (*Artemia* sp.) are used as the major food source for many cultured marine finfish larvae. It is known that the fatty acid composition of *Artemia* nauplii can vary between strains and also from one batch to another within the same strain. To date, DHA has been detected only in very small amounts in *Artemia* (Léger et al., 1986; Webster and Lovell, 1991; Dhert et al., 1993), nor does it accumulate in the eyes of *Artemia* (Navarro et al., 1992). Due to the general lack of 22:6($n-3$) and frequently low levels of 20:5($n-3$) in *Artemia* nauplii, enrichment techniques with oil emulsions are used for enhancing the EFA content of *Artemia* (Watanabe and Kitajima, 1983a; Watanabe et al., 1983b; Léger et al., 1986; Léger et al., 1987; Léger and Sorgeloos, 1991).

However, adequate enrichment of *Artemia* with DHA is difficult as has been shown during the rapid catabolism of this fatty acid in *Artemia franciscana*, the most widely used brine shrimp species in aquaculture. As a consequence, the level of DHA in enriched *Artemia* is unstable, especially when the animals are maintained under starving conditions, (Danielsen et al., 1995; Triantaphyllidis et al., 1995). This has important implications during early first feeding of marine cold water larvae when *Artemia* may reside for more than 2 days in the tanks before being eaten. Such a long delay between enrichment and the larval consumption of pray can severely reduce the nutritional value of the *Artemia*, in particular its content of DHA. The recent finding of the ability of a population of *Artemia sinica* (an *Artemia* species from the P.R. China) to accumulate DHA during enrichment (Dhert et al., 1993) and maintain a relatively high level of DHA during starvation provides a tool to study the metabolic pathways of DHA in different strains of *Artemia*, and may have important implications for larval cultivation of both warm and cold water species. The present study compares the metabolic fate of DHA in two *Artemia* species following enrichment with emulsified lipids and subsequent starvation at various temperatures.

2. Materials and methods

Two *Artemia* species, *A. franciscana* from Great Salt Lake, Utah-USA (ARC No. 1311, INVE Aquaculture N.V./S.A., Belgium) and a population from *A. sinica* (ARC No. 1188: bisexual population collected in Yimeng, P.R. China; Triantaphyllidis et al., 1996), were enriched with emulsified lipids and starved at different temperatures. The enrichment diets, based either on triglycerides (ICES 30/4/C/3; ICES, 1994) or on ethyl esters (Em 50), contained high levels of DHA and relatively low levels of EPA (Table 1).

In two parallel experiments both species were enriched with the ICES emulsion followed by a starvation period at 12.2°C. In an additional experiment both species were enriched with the same emulsion and the nauplii were starved at 6.1 and 22°C. The fatty acid composition and the DHA content was followed during both the enrichment and starvation periods. Samples were taken for fatty acid analysis every 6 h during enrichment and every 24 h during starvation. The enrichment and starvation data at 12.2°C were confirmed with both species in one experiment with the Em 50 emulsion. Samples for fatty acid analysis were taken before and after enrichment and after 6, 12, 18, 24, 48 and 72 h of starvation.

The hatching, enrichment and starvation procedures were standardized and followed in all experiments. *Artemia* cysts were incubated in 1-l conical glass tubes filled with filtered seawater (34 ppt), at a density of 1 g cysts l^{-1}. The glass tubes were placed in a water bath (28°C), continuously illuminated and vigorously aerated to maintain $[O_2] >$ 2.5 mg O_2 l^{-1}. After hatching the nauplii were siphoned out of the tubes, washed with filtered seawater and transferred to 1-l conical glass tubes for 24-h enrichment at densities ranging from 194 to 222 individuals (ind) ml^{-1}. The enrichment diet was added at the start of incubation (time 0) and again 12 h later (both times 0.3 g l^{-1}).

After enrichment the dead nauplii were removed and the live nauplii were sieved, washed with filtered seawater and transferred to glass tubes (200 or 1000 ml) at densities ranging from 176 to 211 ind ml^{-1} and starved (one tube for each sample).

During the starvation period (0–72 h) the glass tubes were maintained in a water bath placed inside a fridge, at temperatures of 6.1 and 12.2°C, or at room temperature (22°C). The lowest temperature (6.1°C) was used because *Artemia* is often kept at low temperatures (6–7°C) before the nauplii are added to larval tanks. The other two temperatures are commonly used during first feeding of marine cold water larvae (10–14°C) and marine warm water larvae (> 20°C). The survival rate of the nauplii was

Table 1
DHA, EPA and $n-3$ HUFA [a] (mg g^{-1} dry weight) concentrations of the experimental emulsions

Experimental emulsion	DHA	EPA	DHA/EPA	$\Sigma(n-3)$HUFA
Em 50 [b]	452	67	6.7	551
ICES [c]	199	49	4	262

[a] Fatty acids having three or more double bonds and a chain length of 20 or more carbons.
[b] Experimental emulsion of an ethyl ester concentrate.
[c] ICES 30/4/C/3, ICES (1994).

followed throughout the experiments by counting the number of nauplii in 12 subsamples (0.5 ml) taken before enrichment (time 0) and before each sampling, both during enrichment and starvation. Low densities of *Artemia* (< 60 ind ml^{-1}) were also used to examine whether defecated food-particles were resuspended and ingested by the animals. This was examined by looking at the differences in the fatty acid content of the nauplii enriched with the same emulsion, starved at the same temperature but at high and low densities, 176–211 ind ml^{-1} and 38–59 ind ml^{-1}, respectively.

The amount of DHA found in the gut of *Artemia* during enrichment and starvation at 12.2°C was estimated by taking two parallel samples from the same tube. One of two samples was sieved, washed in distilled water and directly transferred to vials for fatty acid analysis. Animals from the parallel sample were transferred to a suspension with yeast (*Saccharomyces cerevisiae*, > 1000 cells μl^{-1}), which completely lacked polyunsaturated fatty acids (PUFA), (Oosthuizen et al., 1987). The animals were allowed to

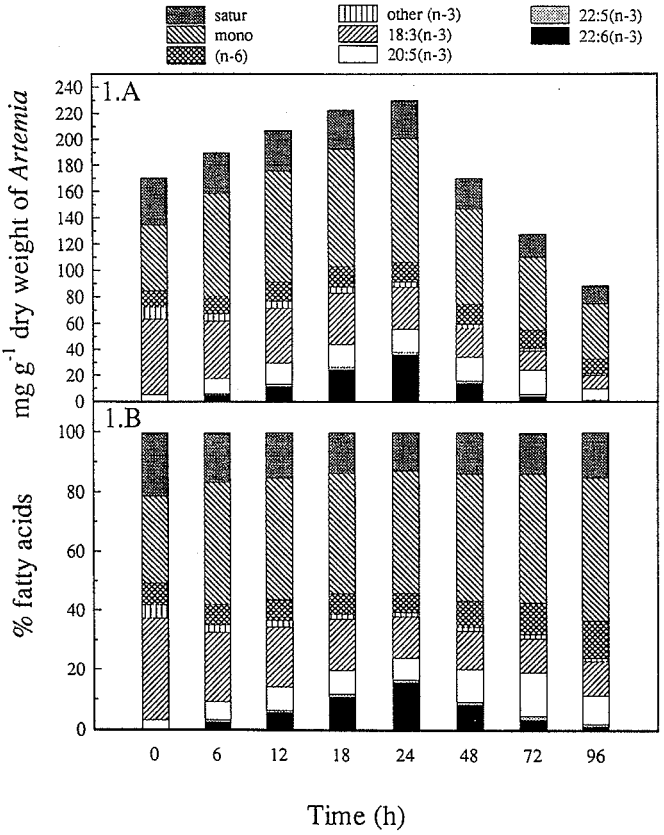

Fig. 1. Fatty acid profiles (A and C: mg g^{-1} dry weight, B and D: % of total fatty acids) of *A. franciscana* (A and B) and *A. sinica* (C and D). Values are shown for newly hatched nauplii (time 0), during enrichment (0–24 h) with the ICES emulsion and during starvation at 12.2°C (24–96 h).

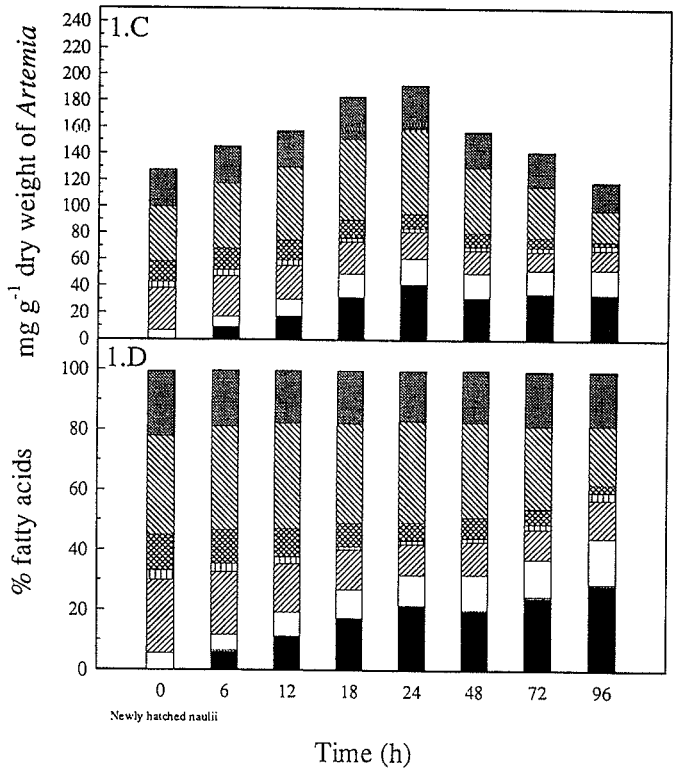

Fig. 1 (continued).

feed for 1 h 45 min, which is longer than the gut passage time under these conditions (Coutteau, 1992), then sieved, washed in distilled water and transferred to vials for fatty acid analysis. The content of DHA in the gut was estimated as the difference between the first and the second samples. The gut passage time of both species at 12.2°C was confirmed from microscopical observations.

Before each sampling, the aeration was stopped (< 3 min) and dead animals were siphoned out of the tubes before the nauplii were sieved, washed in distilled water and transferred to vials, flushed with N_2 and kept in a freezer before further analysis. From this material (199–443 mg wet weight), 3–5 replicates (10.7–29.3 mg) were weighed and the dry weight of each sample was determined after 24 h at 60°C.

At the Artemia Reference Center, the fatty acid composition was determined following a modified procedure of Lepage and Roy (1984). The FAMEs (Fatty Acid Methyl Esters) were prepared following a direct acid-catalyzed trans-esterification without prior extraction of total fat, on samples ranging from 34–133 mg dry weight. An internal standard 20:2($n-6$) was added prior to the reaction. The FAMEs were extracted with hexane. After evaporation of the solvent the FAMEs were prepared for injection by

redissolving in iso-octane and then injected (0.2 μl) into a column on a Carlo Erba 5160 Gas chromatograph. At SINTEF the lipid analysis was performed according to Rainuzzo et al. (1992a) using a modified method of Bligh and Dyer (1959) for lipid extraction and a method derived from Metcalfe et al. (1966) for methylation. The FAMEs were determined quantitatively by a capillary gas chromatograph (Carlo Erba HRGC 5160) fitted with a Shimadzu-Chromatopac C R3A computing integrator.

The experimental data were tested for statistical significance using one-way ANOVA and Student's *t*-test. In all cases probabilities lower than 0.001 ($p < 0.001$) were considered to be significant. The variance of the data is given as standard error (SE).

3. Results

3.1. Enrichment and starvation at 12.2°C

The quantitative fatty acid contents of newly-hatched nauplii from the two *Artemia* species were considerably different at the start (Fig. 1A and B *A. franciscana*, Fig. 1C and D *A. sinica*). *A. franciscana* contained 25% more fatty acids and almost twice the content of 18:3($n-3$) than *A. sinica*. The latter exhibited a slightly higher content of EPA 20:5($n-3$) than *A. franciscana*, 7.01 and 5.3 mg g^{-1} dry weight, respectively. As a result, ($n-3$) fatty acids were approximately 60% higher in *A. franciscana* than in *A. sinica*. Both species contained very low levels of DHA (< 0.21 mg g^{-1} dry weight).

During the 24-h enrichment with the ICES emulsion both species accumulated considerable amounts of ($n-3$) HUFA. In absolute terms (mg g^{-1} dry weight) *A. franciscana* increased its level of ($n-3$) HUFA 9–10 times, whereas *A. sinica* showed a 6–7 times increase. In *A. franciscana* the DHA level increased from approximately 0 to 36.6 mg g^{-1} compared to 0 to 41.3 mg g^{-1} dry weight in *A. sinica*. In both species the EPA level increased approximately 3 times during enrichment and both strains demonstrated DHA/EPA ratios close to 2.1. The levels of 18:3($n-3$) decreased by 45% and 36% compared with the level in newly-hatched nauplii in *A. franciscana* and *A. sinica*, respectively. The content of ($n-6$) fatty acids increased by 17.6% in *A. franciscana* whereas it decreased by 27% in *A. sinica*. The level of monounsaturated fatty acids increased in both species (*A. franciscana*, 47% and *A. sinica*, 35.8%) but the level of saturated fatty acids was little changed during enrichment.

The relative proportions of the different fatty acids (% of total fatty acids) during enrichment are illustrated in Fig. 1B and D. The percentage levels of DHA and EPA increased in *A. franciscana* from 0–15.9% and 3.1–7.5%, respectively. In *A. sinica*, the respective relative increases of DHA and EPA were 0–21.5% and 5.5–10.1%. In *A. franciscana* the level of ($n-3$) PUFA was reduced from 39% in newly hatched nauplii to 15.3% after enrichment. At the same time, the proportion of monounsaturated fatty acids increased from 29.3% to 41.0%. In *A. sinica* the proportion of ($n-3$) PUFA was reduced from 27.7% to 11.6%. In both species the proportion of 22:5($n-3$) was $< 1\%$.

The fatty acid composition during starvation (24–96 h) exhibited considerable differences between the two species, primarily with respect to the level of DHA, but also for the total amounts of fatty acids, ($n-3$) PUFA and ($n-6$) fatty acids (Fig. 1A and

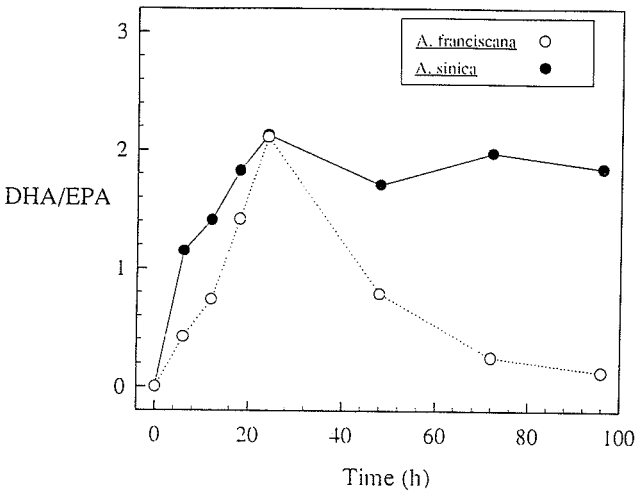

Fig. 2. Comparison of the DHA/EPA ratio in *A. franciscana* and *A. sinica* enriched with the ICES emulsion (0–24 h) and during starvation (24–96 h) at 12.2°C.

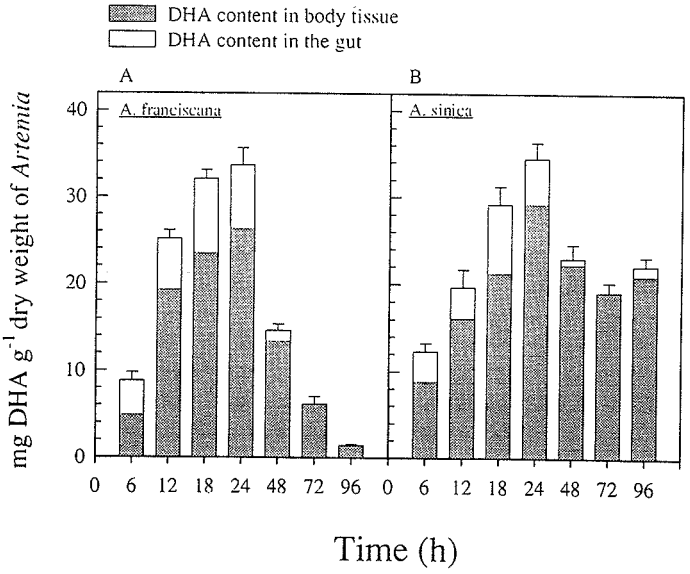

Fig. 3. DHA content of body tissue and gut of *A. franciscana* (A) and *A. sinica* (B) during enrichment with the ICES emulsion and subsequent starvation at 12.2°C. Total length of each bar represents the total amount of DHA in the nauplii (±SE), whereas the white part of the bar shows the gut content. All values are the mean of three replicates.

B). Based on mg g^{-1} dry weight values, half of the total fatty acids and half of the $(n-3)$ PUFA were lost in *A. franciscana*, and most individual fatty acids followed the same pattern. *A. sinica* lost 24% of the total fatty acids, 18.2% of the $(n-3)$ PUFA and 70.4% of the $(n-6)$ fatty acids. The DHA content in *A. franciscana* and *A. sinica* decreased by 97 and 17.5%, respectively.

In *A. franciscana* the percentage levels of DHA were rapidly reduced (Fig. 1B), whereas the relative proportions of both DHA and EPA increased in *A. sinica* during starvation (Fig. 1D). At the end of the starvation period the relative proportions of DHA and EPA was 1.3% and 9.4% in *A. franciscana* and 28.5% and 15.4% in *A. sinica* (Fig. 1B and D). In *A. franciscana* the $(n-3)$ HUFA constituted 11.6% of total fatty acids

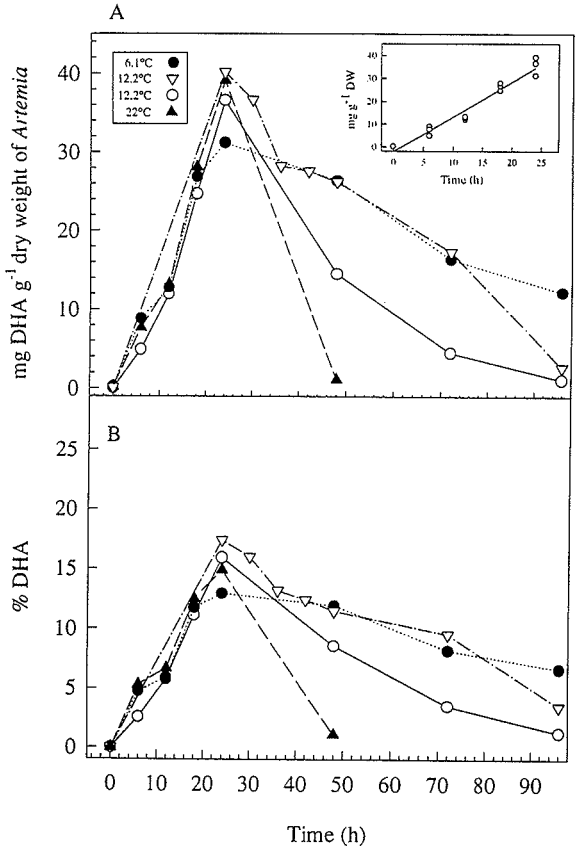

Fig. 4. Retention of DHA during and after enrichment in *A. franciscana* (3A, B) and *A. sinica* (3C, D). Each plot represents one enrichment experiment from which the animals were transferred to starving conditions at the given temperatures. The ICES emulsion was fed during enrichment, except for one experiment (▽) where both strains were enriched with the Em 50 emulsion.

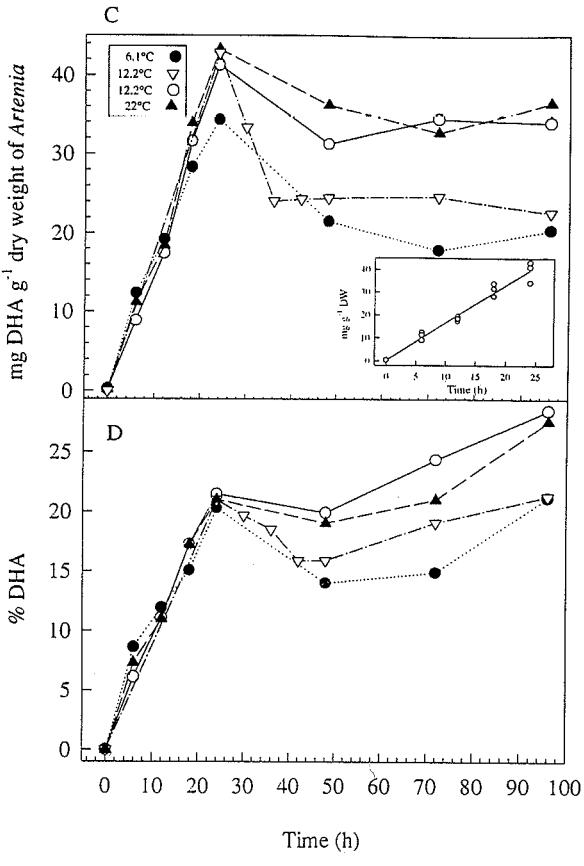

Fig. 4 (continued).

(Fig. 1B), whereas almost half of the fatty acids were $(n-3)$ HUFA in *A. sinica* (44.4%, Fig. 1D). The DHA and EPA content of *A. sinica* gave a high DHA/EPA ratio (1.7–2) throughout the starvation period (Fig. 2) while the DHA/EPA ratio in *A. franciscana* was close to 0.2 after two days of starvation.

Both species were starved at low densities (three parallel groups from each species, < 60 ind ml^{-1}) at the same temperature and over the same periods of time (12.2°C, 72 h). No significant differences in fatty acid composition were obtained between each of the three groups (ANOVA, $p < 0.001$), or between nauplii starved at high and low densities (t-test, $p < 0.001$). In *A. franciscana* the DHA content decreased from 36.6 mg g^{-1} dry weight (after enrichment) to 1.44 ± 0.22 mg g^{-1} dry weight and in *A. sinica* the DHA content remained constant above 33.5 ± 1.89 mg g^{-1} dry weight (mean $\pm$ SE).

3.2. Role of gut content

Determinations of gut contents during enrichment revealed that both species assimilated DHA relatively fast from the gut into the tissue (Fig. 3). At the end of the enrichment period the gut contents of *A. franciscana* and *A. sinica* constituted 27 and 17% of the total DHA content, respectively. During starvation only small fractions of DHA were detected in the gut content ($< 5\%$).

3.3. DHA content in A. franciscana and A. sinica following enrichment and subsequent starvation at different temperatures

In further enrichment studies with the ICES emulsion, nauplii from both strains accumulated DHA at a constant rate during the enrichment period (0–24 h). The accumulation rate of the two species, after regression analysis, was 1.53 ($r^2 = 0.95$) and 1.66 ($r^2 = 0.96$) mg DHA g^{-1} dry weight (h^{-1}), which was not significantly different (*t*-test, $p < 0.001$, for regressions see insets of Fig. 4A and C). The quantitative DHA content in *A. franciscana* (Fig. 4A) and *A. sinica* (Fig. 4C) increased from 0 to 35.6 ± 2.01 and 39.6 ± 2.07 mg g^{-1} dry weight, respectively, (mean $\pm$ SE) in three independent experiments. In one enrichment experiment with the Em 50 emulsion the DHA levels obtained for *A. sinica* and *A. franciscana* were 42.8 and 40.2 mg g^{-1} dry weight, respectively (Fig. 4A and C). The survival rate during the 24 h enrichment period was 82–98% for *A. franciscana* and 68–98% for *A. sinica*.

The temperature had significant effects on the stability of DHA in *A. franciscana* during starvation (24–96 h). At the highest temperature (22°C) the DHA level decreased steadily towards 1.1 mg g^{-1} dry weight over the first 24 h, a reduction of 97% (Fig. 4A). The degradation rate of DHA was slower at the lower temperatures tested. At 12.2°C the DHA content of the nauplii was found to be 1.13 and 2.7 mg g^{-1} dry weight at the end of two independent experiments (96 h) with the two different emulsions, corresponding to a decrease of 97% (Em 50 emulsion) and 93% (ICES emulsion), respectively. At 6.1°C the DHA content was 12.2 mg g^{-1} dry weight, corresponding to a reduction of 61%. The survival rate during the starvation period was $> 85\%$ at all temperatures.

In *A. sinica* a reduction in DHA content was observed but only during the first day of starvation. Thereafter, levels of DHA stabilized and a high proportion of the DHA was retained in the animals at all temperatures (Fig. 4C). The group enriched with the Em 50 emulsion and starved at 12.2°C had reduced DHA content (47%) but only during the first 12 h, following which the DHA level remained relatively constant for the rest of the starvation period (Fig. 4C). For the remaining groups, enriched with the ICES emulsion, the decrease was most striking during the first 24 h of the starvation period (24–48 h). After 72 h of starvation (96 h) 84% of the DHA was retained in *Artemia* starved at 22°C, 81% at 12.2°C and 63% at 6.1°C.

Fig. 4B and D illustrates the relative proportion of DHA (% of total fatty acids) during enrichment and starvation. The DHA level increased during enrichment in both species. In *A. sinica* the DHA level was approximately 8.7% higher at the end of the enrichment period (24 h) relative to that of *A. franciscana*. When both species were

starved, the proportion of DHA in *A. franciscana* decreased steadily at all temperatures reaching < 7% content at all temperatures tested (96 h). In *A. sinica* a moderate decrease was observed during the first part of the starvation period (24–48 h), after which the proportion of DHA reached even higher values than those found just after enrichment (DHA content > 20% of total fatty acids). The survival rate of *A. sinica* was 71% at the end of the experimental period for animals starved at the lowest temperature, and 65% and 76% for animals starved at 12.2 and 22°C, respectively.

4. Discussion

It is well documented that the lipid and fatty acid composition can vary among various geographical strains of *Artemia* and so far high amounts of DHA have not been detected in *Artemia* nauplii (see review of Léger et al., 1986). Furthermore, *Artemia* do not synthesize DHA (Léger et al., 1986) and consequently the nauplii have to be enriched in order to meet the high dietary requirement for DHA in larvae of various marine fish species (Holmefjord and Olsen, 1991; Kanazawa, 1993; Watanabe, 1993). Although *Artemia* enrichment is a common practice in many marine fish hatcheries, limited information exists about the metabolic fate and the retention time of specific fatty acids after enrichment, i.e., period of time between introduction of the nauplii to the larval tank and the consumption of the larvae. Recent findings have demonstrated that the DHA content of *A. franciscana* is unstable after enrichment and that the reduction of DHA is highly correlated with the temperature. During a 24-h starvation period, the DHA content of *A. franciscana* was reduced by more than 92% at temperatures > 25°C (Danielsen et al., 1995). Moreover, in seven *Artemia* populations examined by Triantaphyllidis et al. (1995) the DHA level decreased linearly with time in the populations studied and no differences, in terms of stability of DHA during starvation, were recorded. These findings were confirmed in the present study, demonstrating that, depending on the temperature, 61–97% of the DHA content was lost during 72 h of starvation. These results agree with Dhert et al. (1993) and suggest that *A. franciscana* rapidly metabolizes DHA for energy production, which is also temperature dependent. Furthermore, the results demonstrate that if *A. franciscana* is used as live feed organism and fed to fish larvae at a temperature close to 20°C, the effect of enriching the *Artemia* with an emulsion high in DHA has little effect if the *Artemia* are not ingested by the larvae during the first 24 h after enrichment.

The composition of fatty acids during starvation suggest a different pattern of fatty acid utilization in *A. franciscana* and *A. sinica*. During the enrichment period, the two species accumulated DHA at equal and constant rates and reached almost the same levels of DHA after 24 h enrichment. During starvation, the reduction of DHA in *A. franciscana* was correlated with the temperature, whereas an inverse relationship with temperature became apparent for *A. sinica*. In all experiments the DHA level in *A. sinica* decreased only during the first part of the starvation period (Fig. 4C) after which it remained relatively constant. In relative terms (% DHA of total fatty acid), the DHA content increased towards the end of the starvation period (Fig. 4D), indicating that fatty acids other than DHA were utilized as energy sources. In one starvation experiment

samples were taken regularly at 6-h intervals during the first 24 h of the starvation period (starvation at 12.2°C, emulsion Em 50). These results suggest that the reduction of DHA in *A. sinica* takes place during the first 12 h of starvation. This reduction is likely related to the evacuation of the gut content, which constitutes as much as 17–27% of the total DHA content in the nauplii.

The high and constant levels of DHA in *A. sinica* during starvation together with a DHA/EPA ratio close to 2 make this *Artemia* species of special importance to the cultivation and first feeding of marine fish larvae. Marine copepods from the coastal waters of Norway are often used as first feed for both halibut and cod larvae giving good survival, pigmentation and growth (unpublished results). Among the copepod species examined the DHA level slowly decreases during starvation (8–10% day^{-1}), whereas the DHA/EPA level usually increases from 1.8 to 2.2 during 3–4 days of starvation (unpublished results). These are similar observations to those reported here for *A. sinica*, i.e., a relative increase in % DHA towards the end of the starvation period; this suggests that fatty acids other than DHA are preferentially utilized as an energy source. These findings make this population from *A. sinica* a most suitable live food organism for fish larvae with high requirements for DHA, such as halibut and cod. Moreover, this population may open up new opportunities to improve the nutritional value of *Artemia* as a live food organism. It should be emphasized that this DHA retaining ability is so far only found in this particular population (ARC No. 1188) and not in other population within the same species (Triantaphyllidis et al., 1995).

During enrichment with emulsified lipids, the fatty acid content of *Artemia* correlates well with the fatty acid composition of the enrichment diet (Léger et al., 1986; Léger et al., 1987, Dhert et al., 1993). It was therefore surprising to see only minor differences in DHA and DHA/EPA ratio in both species of *Artemia* following treatment with the two emulsions which had different contents of DHA (Table 1). The DHA levels obtained after enrichment with the Em 50 emulsion and after 72 h starvation were not significantly different from those obtained with the ICES emulsion ($p < 0.001$).

In conclusion, the degradation rate of DHA in *A. franciscana* was dependent on temperature, whereas this was not the case for *A. sinica*. In this particular population of *A. sinica* (ARC No. 1188) the DHA level decreased slightly during the first part of the starvation period, but remained relatively constant thereafter. After 72 h of starvation *A. sinica* still had a relatively high content of DHA, levels which are considered to be appropriate to meet the DHA requirements of marine fish larvae. This is not possible with *A. franciscana* because of the fast DHA degradation during starvation. The availability of a DHA-retaining species (*A. sinica*) and a DHA-degrading species (*A. franciscana*) offers a unique opportunity to further investigate the metabolic pathways of DHA and possibly reveal methods to improve the DHA enrichment in *Artemia*.

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